ORIGINAL ARTICLES

EVIDENCES OF TUBERCULOUS INFECTION IN PEOPLE DYING OF OTHER CAUSES THAN TUBERCULOSIS *

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THE length of time of survival of the tubercle bacillus in the human body, a debatable problem since the work of Naegeli and Von Behring, respectively, resulted in a controversy near the beginning of the century. There was no question about Naegeli's observations, which have been supported by Orth, Beitzke, Opie, Schurmann and others regarding the presence of calcified lesions in practically all lungs of people dying of other conditions than tuberculosis. Whether they were "healed," as many thought, or how many contained living bacilli, were two phases of the problem that were not soon solved. But as time passed the infection rate for most countries gradually decreased, due largely to preventive measures. As a result, the number of such calcified lesions has decreased until in many districts today no more than half of the lungs contain them.

Von Behring's theory regarding the prolonged endogenous progession from childhood to adult life, however, was not so certain. The theory of bovine origin was surely not borne out in any more than a small percentage of cases, but Von Behring's ideas could not be treated lightly. As with so many problems in medicine there was much truth in his contention; the difficulty was apparently due to an incomplete knowledge of all conditions. Doubtless Von Behring actually saw some childhood infections ripen into disease in adult life; but the life time span of the disease is not the common type of endogenous progression. The span of disease rarely extends from childhood to adult life; often extends from childhood to puberty, or from puberty to college age. Many times, particularly in Naegeli's time, there was complete healing in childhood and reinfection in adult life.

The solution of the problems arising out of the apparent paradox was sought by every available means. The first efforts were devoted to seeing how many old lesions described by Naegeli contained bacilli. By inoculating the material into animals, Rabinowitch reported finding living bacilli in nearly half the calcified lesions and in about two-thirds of soft or chalky lesions. Loomis, Schmitz, Kurlow, Wegelin, and Lubarsch obtained comparable results. Opie and Anderson, however, reported positives in only about 30 per cent of all cases. They made pertinent observations also, overlooked by their predecessors, that most soft apical lesions contained tubercle bacilli, but rarely did they find the bacilli in focal calcified lesions elsewhere unless the apical lesions also contained them. To support these findings they reported 45 per cent positive results in lung tissue away from all focal tubercles, apparently bearing out the contention of Theodore Smith, Weichselbaum and Bergel that bacilli can live for long intervals in the lungs without causing any tissue reaction. Herbitz, McConkey, MacFadyen, Loomis, Pizzoni, Spengler, Straus, Wang and others reported similar observations for lymph nodes free of tubercles.

Recently Feldman and Baggenstoss reported the surprising positive findings of only four per cent of focal tubercles in children. Much of their material, however, was shipped to them in borax which may have been detrimental to some of the bacilli. Besides, when so many were selecting the material, there would not be a tendency to uniform sampling. Many of the less dense lesions may have been overlooked. The disparity, however, is certainly not entirely on such a technical basis. The facts seem to point more to a different type of material than was used before. Tubercles in children perhaps differ from those of older people. When such tubercles become encapsulated, most of them go on to healing and rather early sterilization. But if we accept their results at their face value, there is still the unsolved problem of lesions that produce disease later in life.

From pathological studies, Birsch-Hirschfield in 826 accidental deaths, reported that 20.7 per cent had tuberculous lesions with 4.2 per cent of them active. Reinhard found 36.1 per cent of the lesions "not healed" of 360 adults; Hart found 7.2 per cent active lesions in 573 soldiers; while the largest series of all was that of Robertson, who found 4.05 per cent active lesions in the 2.69 per cent with tuberculosis as the principal cause of death in 3306 autopsies at the Mayo Clinic. This work is significant because it is on a large series and the patients were representative of the whole country.

Clinically there have been numerous reports, most of which are well summarized by Sayé. Active disease (adjudged largely on x-ray examination), ranges from less than one per cent in America and many parts of Europe, to eight per cent in some parts of China. Recently Tice and associates found 4.3 per cent in a survey in a heavily infected district in Chicago.

As evident from the figures cited, as well as many more reports in the literature, there is a great deal of discrepancy in the findings, irrespective of the source or branch of science used to obtain them. They are perhaps due to differ-

* Read before the California Trudeau Society and the California Tuberculosis Association, Los Angeles, April 10, 1942.

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ence in concept, difference in material from place to place, and especially from one era to another, and differences in training for a particular objective. For example, as Naegeli's technique improved, his percentage figures rose for positive findings from 75 to 90, to 97 and 98 per cent respectively. In fact, most people in that time must have had gross infection. Obviously there are less infections and less severe infections today than in Naegeli's time. With Naegeli's best technique today, it would not seem possible to find more than 70 per cent of our cases positive. The post mortem x-ray adds to the ease of finding very small lesions.

Another discrepancy in the early reports is in "active" lesions. Most pathologists found four to five per cent of their material positive, but here again figures vary widely; as pointed out by Opie, for clinical appraisal, the differences between latent and clinical tuberculosis have "no other basis than the limitations of diagnostic methods and the tendency of tuberculosis to proceed to recovery." The same indefinite line of demarkation also exists between the concepts of pathological activity. Some may call a lesion active with a thin capsule, others only with perifocal giant cells, while still others might demand soft caseous lesions. Roentgenological criteria of activity is even less accurate than either pathological or clinical.

One absolute criterion alone remains, viz: living tubercle bacilli *in* the lesions. Even this must be supported by collateral pathological findings such as a thin capsule, giant cells in or near the capsule, otherwise the bacilli may be from recent exogenous sources and may not lead to disease. *The presence of lesion-producing bacilli, however, is prima facie evidence of potential disease.*

Due to improvements in technique and by using every available means at our disposal, it was thought feasible and justifiable to try to clear up some of the discrepancies existing in the reported results of the past, and to discover any clews with respect to the exacerbation of the disease.

The present study was, therefore, planned to achieve that aim, although it was nearly ten years before it was an unqualified success. During the intervening time, several different attempts were made with meager or inconclusive results. The material was always difficult to obtain in sufficient quantity. Such a result is to be expected when it is necessary to depend upon people having no special interest in the work. Of 45 acceptable cases of these various attempts, however, five had positive cultures (11.1 per cent), a result that was an encouragement to further effort. The various attempts to carry out the work did help to develop a trained organization and a standardized technique which has contributed largely to the success of the venture. The work was finally reorganized about a year and a half ago with new objectives, a stabilized personnel, and an assurance of abundant material.

OBJECTIVES

The prime object of the work was to attempt to discover the incidence of infection as it exists today in people apparently well; the incidence of the disease in ill people but not having had a diagnosis or any recognized symptoms of tuberculosis; to see if anything could be determined regarding the mode of development of the disease; to see if the development of the disease could be related to any state or condition of the host with regard to age, race, sex, occupation or intercurrent diseases; to see if any means might be used during life to discover dangerous lesions by studying of histories, and from physical, x-ray and laboratory findings. In addition it was desirable to find if possible the relation of the presence of tubercle bacilli to the age and character of the lesions and to other disease processes and to pathological evidences of active tuberculosis near and away from the focal lesions. Finally, it was desirable to find the comparative value, if possible, of different culture methods, acid-fast staining, and fluoroscopic microscopy in the identification of the presence of living bacilli in tissues.

FACILITIES AND ACKNOWLEDGMENTS

As might be inferred from the preceding discussion, it was most important to have an uninterrupted flow of material, suitable laboratories with adequate supplies, and corps of trained workers.

The laboratories at the Municipal Tuberculosis Sanitarium afforded the facilities for carrying out the work, including bacteriological, pathological, x-ray equipment, and the materials necessary for these various operations. These facilities were made available by the authority of the Board of Directors, Dr. Frederick Tice, President; Mr. Harry Reynolds, Treasurer; Dr. Richard Davison, Secretary, and Dr. Leo M. Czaja, Superintendent of the Institution.

Much of the material was furnished by the Cook County Hospital Pathological Laboratory with the coöperation of Drs. Jack D. Kirshbaum and William P. Mavrelis. The Coroner's Pathological Laboratory and the Research Hospital Pathological Department also furnished much valuable material for study.

Special aid was also obtained from the Tuberculosis Institute of Chicago and Cook County.

The technical part of the culture work was carried out or supervised by Miss Asya Stadnichenko until she was forced out by illness and an untimely death. Since then her assistants, Messrs. William I. Lansford and John M. Kleech, and her sister Miss Vera Stadnichenko have carried on with no more interruptions than could be expected following such a misfortune.

The pathological section work has been carried out by Miss Alma Everett and her assistants.

The post mortem x-ray and liaison work has been done by Mr. Tom Cantalancio.

The photographs and illustrations have been

prepared by Mr. William L. M. Martinsen.

METHODS

The method of procedure was so organized that no two groups could know the results of any other until the final reports were obtained. The lungs were removed and x-rayed; the various lesions suspected of being tuberculous were charted on a stamped outline of the lungs. Each lesion was given a number. Any extra-pulmonary tuberculosis (and other disease) was also recorded. The various tuberculous lesions were then removed with sterilized instruments and placed in sterile bottles bearing the proper number of the tubercles on a label. The culturing process was carried out with sterilized equipment. The individual lesions were removed from the bottles by sterile forceps; the excess tissue was trimmed off and cultured for most of the first 200 cases. The practice was discontinued for reasons to be given later on in the work. The tubercle, with a small amount of tissue around, was cut in two with sterile scissors, one-half being placed in formaldehyde solution for section and the other in a sterile mortar and ground into an impulpable powder or magma for culture, smears and animal inoculation. The contents of the covered mortar were taken up in sterile salt solution and treated in the standard manner NaOH, HCl, or C_2H_{204} (and sometimes two of these substances) and planted on two to five culture bottles of three or four different media. The culture medium used was that of Saenz, Loewenstein's, modified by Jensen and Holmes, Wooly-Petrick, and sometimes a modified Sweany-Evanoff medium and Arena's medium. Each soft or semi-soft lesion was worked up separately and cultured. At least one guinea pig was used on the fresh lesions for each case. Old dense lesions of the same side were frequently pooled. The cultures were observed weekly after three weeks and were not rejected until six months' time had elapsed. The animals were killed and reëxamined after sixty days.

The specimens for pathological section were decalcified and stained first with H and E, but all tissues positive on culture were sectioned, stained and examined for tubercle bacilli. Many times recuts and partial serial sections were made.

The microscopical pathological examination included a detailed description of the pathological formations in the section with a rough estimation of the age of the lesions as outlined in a former study. Owing to the fact that half of the lesion was taken away from culture and usually only one section was examined, the analysis was less accurate than in previous work. As later results show, however, there was an interesting relationship of age to the number of positive cultures. The smears were examined completely for acid-fast bacilli on early type lesions. All old lesions were studied for at least fifteen minutes as routine.

All of this work was done without any knowledge concerning the patient. All of the clinical, pathological and x-ray data was compiled by another team of workers and the various findings finally fitted into a master chart.

EXPERIMENTAL RESULTS

This preliminary report involves only the first 300 completed cases of 800 cases already partly worked up.

Table I shows the division of the cases on the basis of the presence of, or the type of calcification. There were 37 lungs without any calcified lesion at all; and 51 in which the lesions were too small to divide successfully with scissors. The total of 88 unstudied specimens was 29.33 per cent, leaving 212 (70.67 per cent) that were studied. There were 23 cases in which no lesions were found having age characteristics suitable for analysis. Some were silicotics; others had "silicotic fibrosis" or other evidence of pneumoconiosis. Some were chondromas, or other calcified pathological lesions than tuberculosis; others were "fibroid caps"; while a few were not suitable for age analysis at all.

The results of search for evidence of tubercle bacilli in the 212 cases studied, are shown in table II. Acid-fast bacilli and positive cultures are compared and both are combined. There was 10.38 per cent positive for acid-fast bacilli, against 16.53 per cent positive cultures. Both together gave a positive finding of 20.75 per cent. There were 14 cases in which both were found positive; 21 positive on culture, but negative on smear; and 8 positive on smear but negative on culture. The total number of colonies averaged about ten times as many as there were acid-fast bacilli found. Since there was about ten times as much material used for cultures as was used for smears, the results were fairly comparable. There was not always agreement, however, of pathological lesions with positive culture and smears. It must be remembered that the parts saved for section were not cultured, and vice versa, and that many times tubercles have "budding" colonies only on one side. Furthermore, the sections made involved only one small portion of the half for section, while the culture represented practically all the half that was cultured. It may also be possible to have positive cultures without evidence of recent tubercle formation. Bacilli may possibly live for some time entirely encapsulated. The results, however, seem to indicate that the average time of survival isn't long, without producing tissue reaction.

In table III are arranged the 189 cases on which age analysis was possible on at least *one* lesion, with the positive findings recorded of the youngest lesion immediately below the percentages in the third line. In line four are cases having slight silicosis and line five are the corrected figures.

Outside of the cases having one or more silicotic lesions, the bacilli disappeared from tubercles rather rapidly and at a regular rate. Although

a few cases seem to show life in tubercles up to 10 years, the evidence indicates that enclosed lesions do not retain living bacilli long after two years and many become sterile after one year. The apparent persistence of the bacilli is due to the "spreading," "overflowing," or otherwise progressing lesions. Sometimes it may be confined to only one tubercle and even only a small part of one tubercle, but in such cases the bacilli escape the tubercles to lie dormant in the outer capsule or in the tissue beyond for long, or to form into new colonies which become encapsulated. The fact that we haven't always found the fresh colonies does not argue against their existence, because we only observed a small percentage of the surface of any one tubercle. One-half was cultured and a section was made of the other half that represented only a small per cent of all the surface.

The question of what causes the bacilli to survive or what causes the tubercles to weaken and disseminate the bacilli, is largely unanswered. It was at first suspected that silicotic fibrosis may be a dominant cause, but although high, a higher percentage of positives resulted from "ruptured" or "overflowing" lesions than from those having silicosis or "silicotic fibrosis." The only significance of small areas of silicosis appears to be that they afford a "hide out" for the bacilli. Disease comes only after a critical quantative threshold is reached.

Positive findings of pathologically active tuberculosis were present in three cases (1.41 per cent) of the 212 cases studied. One case was an old healing fibroid, another a progressive fibrocaseous infiltrate, and a third a miliary and acinous-nodose progressive tuberculosis in a child. This figure is low, because obvious and advanced tuberculosis was not given to us, and represents quiescent lesions only. In addition, there were 22 (10.38 per cent) cases in which there were definite evidences of progress of the disease around one or more of the calcified lesions as an overflowing or otherwise slowly progressive process. Of these 22 cases, 12 (54.54 per cent) have positive cultures. Only two of these lesions were of a silicotic nature. The cause of occult progression is still enigmatic. The bacilli may have gained more virulence; the host may have a temporary depression in resistance or an accident, disease, or drinking bout at a critical moment in the existence of the lesion.

The group of silicotic cases was of absorbing interest, not only from the standpoint of industrial medicine but more important from the mechanism of survival of the bacilli. The results are charted in table IV. There was one case of second-stage (diffuse type) silicosis, and two cases of typical first-stage silicosis, all of which were positive. Practically every lesion was positive with numerous colonies and acid-fast bacilli in smear. There was one case of silicotic fibrosis rather marked in some of the hilum lymph nodes, but no cultures were found positive. There were 11 cases with a few whorls of silicotic fibrosis in one or more lesions, two of which were positive, and 15 cases of similar type lesions except there was either much coal or iron pigment, or evidence of tuberculosis caseation present in addition. Five were positive. In 30 cases having slight or moderate silicosis 10 were positive (33.3 per cent).

The interesting feature was that in *none* of the "silicotic" lesions could there be found any recent signs of tuberculosis activity. The bacilli seemed to harbor in the old nodes and produce slight caseation and an "egg-shell" calcification but no cellular reaction. Perhaps the defense mechanism may have become exhausted within the nodules or the bacilli may be able to live without increasing much in numbers (until later in the disease). While the bacilli may survive in the lesions, there do not appear to be any more cases develop active tuberculosis than in other cases. The presence of bacilli, however, is admittedly a threat, but there was a lower percentage of positive findings in the silicotic types than in those showing incidence of a progressive disease. There seems to be a considerable degree of silicosis necessary before a progressive tuberculosis can develop.

Case No. 293, a first-stage silicosis, had lesions that were hard and fibrotic with very little tuberculosis in spite of strongly positive cultures. Case No. 169 was more tuberculous but still not caseous in an 84-year-old man. Had he lived twenty more years he might have died of silicosis.

Several things seems certain: Silicosis can develop without tuberculosis, but when tubercle bacilli come in they tend to remain for long intervals without eliciting any proliferative tissue activity or without appearing to grow extensively until the fibroid tissue "hideout" for the bacilli becomes extensive. Then the bacilli seem to be able to come out in the open and produce disease. All the implications of these observations, however, must await a final review of the whole series of cases before any final judgment can be passed, if then.

The same phenomenon of "dormant" bacilli seemed to exist in cancer tissue. Of five cases in which cancer tissue was found, two (40 per cent) were positive. The same principle may operate as in silicosis, viz: bacilli may survive in cancer tissue, but not grow. There is perhaps no tubercle-forming tissue in the cancer.

In a few cases of old fibroid lung tissue there also seemed to be living bacilli without tubercle. This fact was pointed out by Opie when he obtained growths in lung tissue around tubercles more than in the lesions themselves. It raised the question in his mind whether most of his infections were not coming from outside sources and not from the tubercles. While there is no doubt about a "tissue immunity" existing around tubercles or an "exhaustion" of the resisting forces as the old focus is approached, it cannot explain all the many lesions within the capsules nor the increasing of positive findings as the lesions de-

crease, in age. As in silicosis, in some cancer cases and some old fibroid lesions, there is a nominal percentage of bacilli from exogenous sources. In the oldest lesion groups there was a small percentage of positive findings. There are still residue infections, however, in the old tubercles that gradually diminish as the years pass. Some die out soon (perhaps as soon as one year) but a few by microscopic extensions persist for as long as five years or longer.

In this preliminary report many important details must be omitted, but a few general observations may be made. For example, there was a strong evidence of exogenous reinfection in 12 cases (5.9 per cent); seven (3.3 per cent) had clear-cut "reinfection complexes." Naturally there were more reinfections than this, but just because there happen to be soft lesions in the apexes and calcified lesions in the bases or at the hilum does not prove exogenous reinfection. Many times definite progression from one lesion to another can be traced (as in x-rays No.'s 225 and 235). One of the problems of this study has been to work out a rational means of tracing such infections in the body. Where the ages of the lesions are not widely different and where living bacilli can be found in the older lesions or the giant cells in the capsules, there is no justification in saying the soft lesion is from exogenous sources. It *may* be, but many times endogenous infection can be established without difficulty. Here again we are forced to wait for the complete and final study.

An important collateral observation was that in cases having signs of generalization, no living bacilli were found in any of the lesions. In two of the cases the lesions were less than two-year types, and two more were less than four. In these four cases giant cells were present in many of the lesions, but no positive culture or animal inoculation was obtained in spite of the fact that from 10 to 20 lesions were studied from each 10 guinea pigs and 242 culture bottles inoculated. It seems to suggest that mild generalization produces an unfavorable environment for tubercle bacilli. The findings are shown in table V.

Another important finding is of special interest to roentgenologists. There were 4.29 per cent calcified focal lesions that proved not to be tubercles, not including calcified bronchial cartilages. Among these, were four chondromas; two osteomas (these may have been very old tubercles); one calcified lipoma; one fibroma; one phlebolith, and one case of ossified fibrous tissue in an old fibroid apex. The last lesion showed ossified blood vessel walls and ossified hyaline connective tissue.

While there were many other minor pathological features of interest, there has been no attempt made to correlate the roentgenological findings or the clinical histories. That interesting data will be given in the complete report, together with a more exhaustive discussion of the results and their significance.

SUMMARY

A study has been made of lesions in the lungs and related lymph nodes of 300 patients dying of other conditions than tuberculosis.

Positive cultures were obtained in 16.53 per cent of 212 cases in which detailed studies were made. Of the same group, only 10.38 per cent showed acid-fast bacilli on smears, but they were not identical, as 21 cases (47.72 per cent of all positives) were positive on culture and negative on smears, and eight (18.18 per cent) were positive on smear and not on culture. Both methods together gave a positive result of 20.75 per cent.

When arranged according to the ages of the lesions there was a positive finding of 80 per cent of lesions less than one year, and a gradual drop of 23.1 per cent at the end of ten years. By deducting cases with silicotic or other interfering lesions, it causes the curve to reach an "irreducible" minimum much sooner. The presence of bacilli in the younger lesions is thought to be due to persistent and "overflowing" lesions, and to silicosis, cancer, etc. In a certain definite number of all lesions, and all "old" lesions, "dormant" bacilli are present from exogenous sources. The conclusions from all of these observations must await more seasoned study.

In seven cases where partial generalization occurred, no bacilli could be found in spite of the fact that in four of the cases the lesions were less than four years, and two had lesions appearing less than two years. It seems to offer a problem for immunologists.

Lesions having "budding" tubercles, "overflowing," or ruptured capsules, or giant cells in or just outside the capsules, contained more bacilli than in any other type.

Silicotic lesions or lesions having slight "silicotic fibrosis," were prone to contain tubercle bacilli more than the average lesions (33.3 per cent). This feature loses its principal significance since it is impossible to estimate the age of silicotic lesions.

Most tubercles gradually heal, beginning after a few months and continuing for seven to ten years, depending chiefly upon the number and persistence of the preceding changes.

There were 4.29 per cent of the "dense" calcified or partially calcified lesions which were not tubercles or silicotic nodules. They were chondromas, osteomas, lipomas, fibromas, phleboliths, etc. It illustrates the need for roentgenograms to study relationships and characters of densities in lung roentgenograms.

Tuberculosis control is a vital part of national defense, according to recent statements of the surgeons general of the U. S. Public Health Service, the Army and the Navy.